

**INFLUENCE OF CULTURAL PRACTICES ON SOYBEAN NECTAR PRODUCTION****T. Smith****A. Catchot****J. Harris****N. Krishnan****Mississippi State University****Mississippi State, MS****J. Gore****D. Cook****Mississippi State University****Stoneville, MS****Abstract**

Soybean nectar contributes to a large percentage of the nectar used in honey production in many areas across the Mid-Southern U.S. The amount of nectar produced in each cultivar may differ considerably. This research was conducted to identify which modern cultivars produce the highest quantity of nectar. The objective of these tests were to identify how soybean cultivar influences nectar concentration in soybean. Tests were conducted during the 2016 growing season in the Hills and Delta regions of Mississippi. These tests evaluated planting date and irrigation in both regions and how they influenced nectar production. Flowers were pulled at peak nectar hours and individually weighed. Samples were centrifuged and placed in cold storage until analyzed by spectrophotometry. A number of varieties such as AG4633 and A5535 produced more sugar in the Hills region of Mississippi. Two varieties, AG3936 and AG3536, produced more in the Delta region. Average amounts of glucose were also observed to be higher in Hills region varieties, while average fructose was more abundant in Delta region varieties. A negative correlation between yield and total amounts of sugar was also observed.

**Introduction**

Soybean production in the state of Mississippi has increased roughly 800,000 acres in the past ten years. With that increase in soybean production, there has been a steep increase in the honey production in the state as well. The major component in the production of honey is nectar. Nectar is a diluted solution of many components, most namely, glucose, fructose, and sucrose. Honeybees gather nectar and add special enzymes to make a complex mixture. The mixture is then placed in honeycombs to let the water in the mixture evaporate. Once the evaporation of the water is complete, the honeycomb is capped and the ripened product is honey.

Honey is a mixture made up of a number of different components. The most notable components are water, glucose, sucrose, and fructose. Other components include: organic acids, ash and other minerals, and nitrogen. Glucose makes up roughly a third of the composition of honey. It is an organic molecule as well as a simple sugar. Fructose is a monosaccharide that makes up a third of honey. Sucrose is a disaccharide that only makes up two percent of honey. The honeybee breaks down the sucrose into glucose and fructose which are the main components needed. Because soybeans are frequently targeted by beekeepers to produce a honey crop in Mississippi, this research is being conducted to see if factors that may influence nectar production can be identified to maximize soybean productivity. If soybean producers can utilize varieties that also benefit beekeepers with no negative economic impact on their operation, it is possible that they can develop a mutually beneficial relationship.

**Materials and Methods****Evaluating varietal effects on soybean nectar production**

This experiment was conducted at two locations in 2016: the R.R. Foil Experiment Station in Starkville, MS and the Delta Research and Extension Center in Stoneville, MS to evaluate the influence of variety on soybean nectar production. Thirteen varieties were arranged in a randomized complete block design with four replications. Individual plots were 4 rows, 50 ft. long and planted on 38 inch centers. Plots were planted in early May at a seeding rate of 8 seeds per foot. Varieties evaluated were: AG3533, AG3536, AG3832, AG3936, AG4135, AG4232, AG4632, AG4633, AG4835, AG5335, AG5533, AG5535, and AG5935.

Samples were collected at R1 and again at R2 between the times of 10:00 AM and 2:00 PM. Five flowers from the upper most fruiting position of 5 random plants were removed. Flowers were cut and placed peduncle down into 200

$\mu\text{L}$  of deionized water in a 1.5 mL Eppendorf tube. Samples were placed in plastic coolers lined with frozen packs and taken to the laboratory. In the laboratory, flowers were weighed individually with a Denver Instrument model P-114 scale after the peduncle and calyx had been removed. Flowers were then placed upside down in a new 1.5 mL Eppendorf tube filled with 200  $\mu\text{L}$  of deionized water. Next, samples were placed into a Sorvall RT6000 Refrigerated Centrifuge and centrifuged at 3000G for 15 min to aid with extraction of the nectar. After centrifugation, the samples were frozen at  $-20^{\circ}\text{C}$  until analysis.

The procedure used to process the nectar samples for total sugar analysis was an anthrone assay. 500  $\mu\text{L}$  of a chloroform-methanol (1:2) solution was added to the sample and vortexed rapidly. Samples were then centrifuged at 8000G for 10 minutes at  $4^{\circ}\text{C}$ . 150  $\mu\text{L}$  of the supernatant was then mixed with 100  $\mu\text{L}$  of deionized water in a new 1.5 mL Eppendorf tube and then mixed rapidly. 500  $\mu\text{L}$  of anthrone reagent was then added to the mixture. The anthrone reagent was made by dissolving anthrone in concentrated sulfuric acid to make a 0.2% solution. Samples were then placed in a dry bath for 10 minutes at  $90^{\circ}\text{C}$ . 200  $\mu\text{L}$  of the solution was then pipetted into triplicates in wells of a Fisher brand micro titration plate. Samples were then placed into a BioTek Synergy H1M plate reader and absorbance was read at 630nm. Each value of the individual sugar was then calculated for the unknown samples from a standard curve of each sugar that was generated in the GraphPad Prism 7 computer program. Standards for each sugar was made using the anthrone assay procedure and calculated at a range of 0, 12.5, 25, 50, 75, 100, 150, and 200  $\mu\text{g/mL}$  of deionized water. Plots were mechanically harvested with a plot combine. Test weight, moisture, and weight were recorded for each plot.

### Results

The amount of glucose was found to be significantly different between varieties and locations (Figure 1). Some varieties produced more glucose in the Delta region compared to the Hills region. Glucose was observed to be significantly higher in the Hills region in the varieties: AG3832, AG5935, AG4633, and AG5535. These varieties produced significantly lower amounts of glucose in the Delta region. Two varieties, AG3936 and AG3536, produced much higher amounts of glucose in the Delta region and were observed to be in relatively low amounts in the Hills region. Many of the other varieties were observed to have no significant differences between the locations.

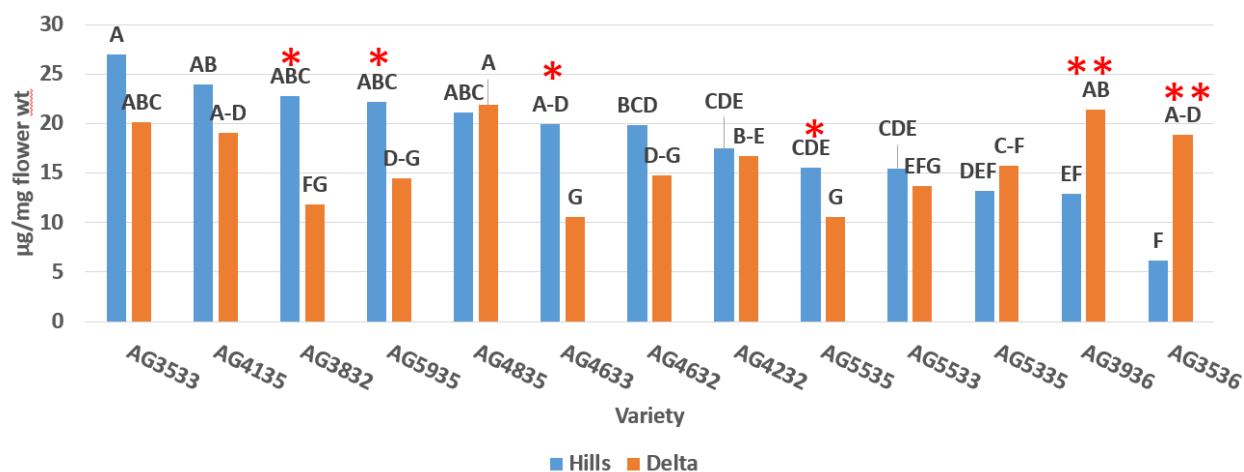


Figure 1. Glucose Interaction of Location and Variety ( $P<0.0001$ )

The amount of sucrose was found to be significantly different between varieties and locations (Figure 2). Some varieties produced more sucrose in the Hills region compared to the Delta region. Glucose was observed to be significantly higher in the Hills region in the varieties: AG3832, AG5935, and AG4633. These varieties produced significantly lower amounts of glucose in the Delta region. Two varieties, AG3936 and AG3536, produced much higher amounts of glucose in the Delta region and were observed to be in relatively low amounts in the Hills region. Many of the other varieties were observed to have no significant differences between the locations.

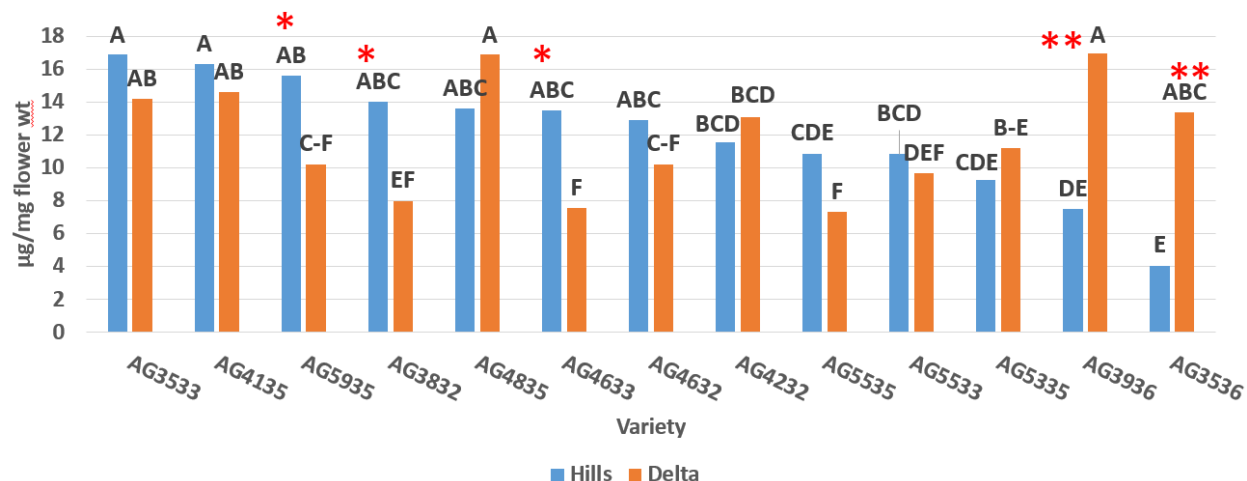


Figure 2. Sucrose Interaction by Location and Variety ( $P < 0.0001$ )

Fructose was not significantly different among most of the varieties that were measured, however, AG5935 and AG4633 were significantly higher in the Hills region compared to the Delta region (Figure 3). Whereas AG3936 and AG3536 had higher amounts of fructose in the Delta region compared to the Hills regions.

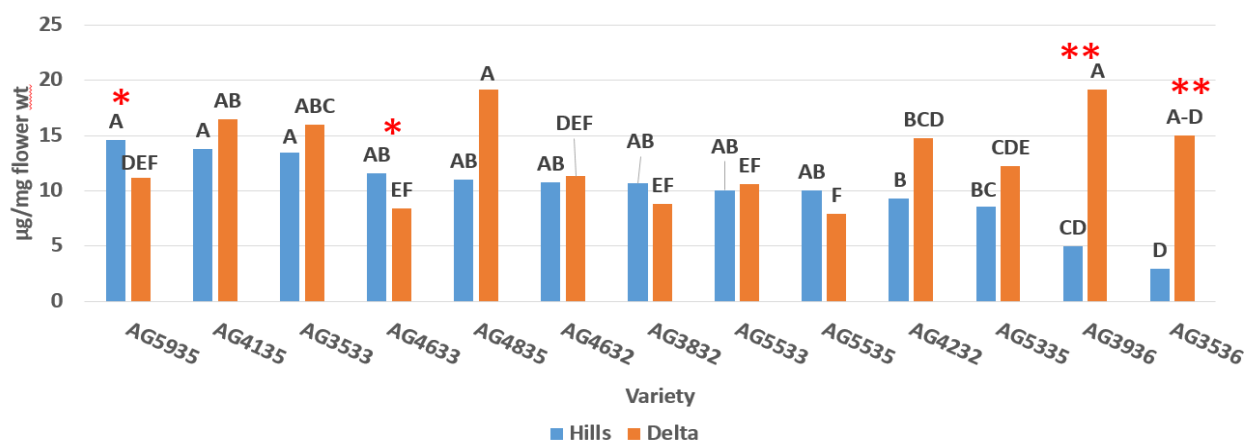


Figure 3. Fructose Interaction by Location and Variety ( $P < 0.0001$ )

Across all varieties, glucose was higher in the Hill region compared to the Delta region. However, there was significantly more fructose found in the Delta region than the Hills region. There was no difference for sucrose between the two regions (Figure 4).

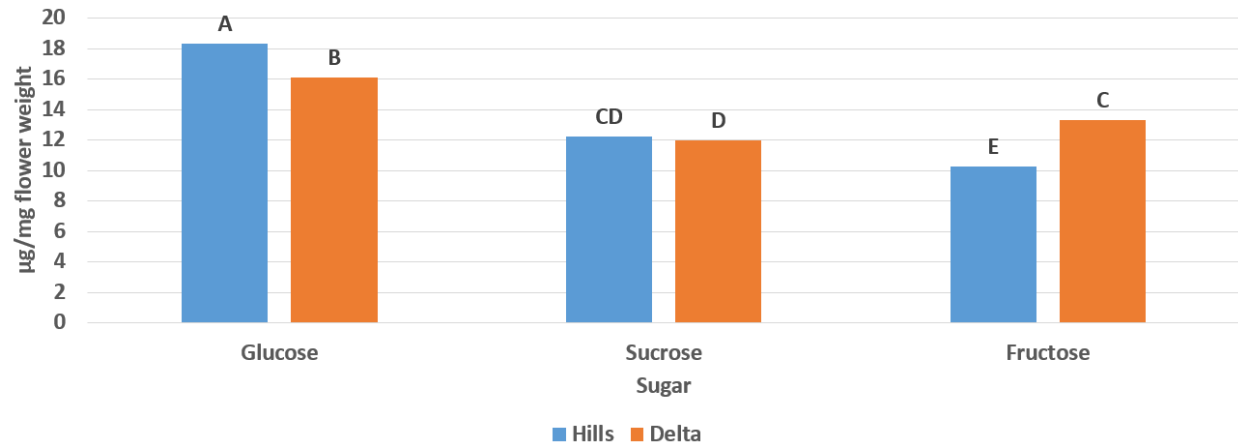


Figure 4. Sugars by Location (P&lt;0.0001)

Sugar type and yield were negatively correlated with one another (Table 1). As yields increased the amount of each of the sugars present were lower compared to varieties that showed lower yields and higher amounts of sugar. The negative correlation of each sugar in the Hills region is not significant and is relatively weak. Whereas in the Delta region, although the correlation is considered moderately weak, it is significant enough to pose further questions.

Table 1. Correlation of Sugar Type and Yield

	Sucrose	Glucose	Fructose
Hills	-.13	-.09	-.22
Delta	-.31	-.31	-.32

### Summary

A variety by location interaction for each of the separate sugars was observed. This was to be expected as some varieties perform better in certain locations and conditions compared to other varieties. There was no significant difference in the amount of sucrose observed across both the Hills and the Delta locations. Glucose was significantly higher in the Hills location, whereas fructose was higher in the Delta location compared to the Hills location. There was a negative correlation between yields of all the varieties and the amount of individual sugar types. The correlation was not significant in the Hills location but is highly significant in the Delta region. The generalization is that due to soybeans being grown in high yielding areas in the Delta region, the amount of flowering positions is much higher. The individual soybean plant is putting more energy into production of flowers and fruit. The amount of nectar production in turn is much lower per individual flower, contributing to the high yields and low amounts of sugar that is being seen in the Delta location.